Incorporating Cell Viability Assays into High Throughput Screening Using the FDSS6000

Introduction

An antagonist assay measures calcium mobilization using a known agonist: receptor model following pre-incubation with a compound meant to block agonist binding. Signal inhibition indicates compound-mediated antagonism. However, inhibition may also result from compound: fluorescent dye quenching occurring inside cells[1)] or compound-mediated cellular toxicity.

Cellular viability assays measure different stages of necrosis (accidental death) or apoptosis, (programmed cell death). Both necrosis and apoptosis proceed through well-defined temporal steps; numerous assays are available to measure various stages of death. For example, early in apoptosis mitochondrial membrane potential dissipates; JC-1 dye (M34152 IVGN, ex488 em529 and 590 nm) measures differences in mitochondrial membrane potential. Other assays for measuring apoptosis are commercially available.

Late stage loss of membrane integrity is common for both necrotic and apoptotic cells.

Materials and Methods

Calcein Blue AM (C-1429, IVGN) diluted in DMSO to 2 mM.

Calcein Blue-AM (ex360 em450) measures cytoplasmic esterase activity, an indication of membrane integrity. The signal is reported as a change in counts per second or area under the curve and is usually linear.

Ethidium Homodimer (E-1169, IVGN) diluted in DMSO to 3.2 mM.

Ethidium Homodimer (ex528 em617) binds DNA in cells with disrupted membrane integrity. The signal reaches equilibrium quickly.

Isopropanol

Hank's/HEPES buffer

Calcein Blue AM Study

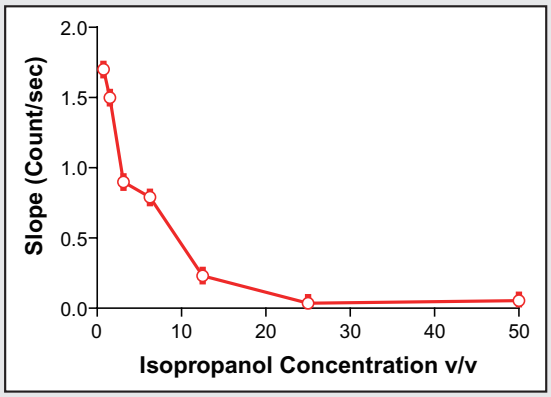

Fig. 1

Media was decanted from 96 well plates and Isopropanol was added in final concentrations ranging from 50 % to 0.75 % v/v. Calcein Blue AM was added to a final concentration of 5 μM in Hank's/HEPES.

FDSS6000 was configured as follows: Excitation 380 nm, UV dicroic mirror (440 nm LP) and emission 465DF30, normal exposure time, and sensitivity set to 4.

Data was collected for 2:30 and was linear (data not shown). The slope was calculated; results are presented in Figure 1:

The results indicate the LD_{50} of Isopropanol is about 6 % v/v.

Ethidium Homodimer Study

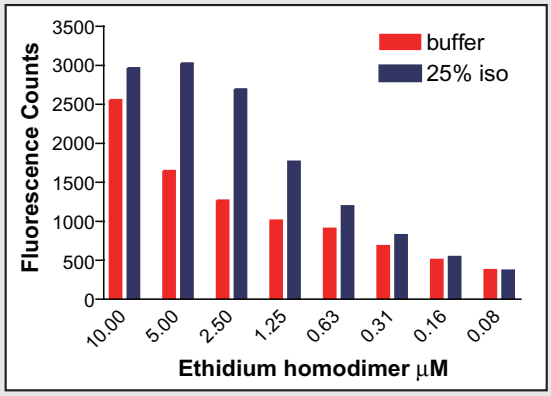

Fig. 2

FDSS6000 was configured as follows: Excitation 480 nm, B dicroic mirror (495 nm LP) and emission filter 645AF75, normal exposure time, and sensitivity set to 4.
A series of wells were treated with either buffer or Isopropanol 25 % v/v. Ethidium Homodimer in dilutions ranging from 10 μM to 0.08 μM were added to buffer-Isopropanol matched wells. The wells were read for one minute and the average signal collected.
The results (Figure 2) indicate 2.5 μM Ethidium Homodimer provides the best buffer to Isopropanol signal separation.

Discussion

The FDSS6000 can be configured to read both Fluo-4 and Calcein Blue AM dyes in the same assay[2]. (Calcein Blue AM is added with agonist). Although not conclusive, a given compound mediating calcium mobilization inhibition and not decreasing Calcein Blue AM metabolism is more interesting than a compound showing inhibition and decreased or no Calcein Blue AM metabolism (loss of membrane integrity, indicative of cell death).
Ethidium Homodimer can be optically paired with Fura-2 dye. The FDSS6000 reads a single wavelength of Fura-2 and Ethidium Homodimer or Ethidium Homodimer is added and the plates rerun following Fura-2 AM ratiometric data collection. (Since the signal is stable only a few readings are collected). Although not conclusive, a given compound mediating calcium mobilization inhibition with little Ethidium Homodimer signal above background is more interesting than a compound showing calcium inhibition associated with Ethidium Homodimer binding (loss of membrane integrity).

References

1. Fluorescence Quenching in Cell-Based Assays Can Be Dye Specific: A Case Study Using Both Fura-2 AM and Fluo-4 AM Calcium Reporter Dyes.
Hamamatsu Photonics Corporation Application Note.

2. Multiplexing Calcium Mobilization and Membrane Potential Assays Using the FDSS6000.
Hamamatsu Photonics Corporation Application Note.

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